# Highly multiplexed protein analysis using NULISAseq™ assays with Illumina next generation sequencing readout

TECH NOTE

## NULISAseq Assays

The NULISAseq assays provide high sensitivity analysis of hundreds to potentially thousands of proteins from a single sample utilizing the power of next generation sequencing as the read out. This white paper describes the recommended process for performing sequencing of the barcoded NULISAseq library on an Illumina sequencer. Additional information and detailed protocols are provided within the ARGO™ HT User's Guide.

The NULISA technology contains two target-specific antibodies each conjugated to a unique oligonucleotide and either a biotin tag or a polyadenylated oligonucleotide which facilitates a proprietary workflow that greatly reduces background and thus increases sensitivity and dynamic range. The resulting purified immunocomplex undergoes a proximity ligation reaction and amplification to create a library of synthetic DNA barcodes that can be sequenced and quantified on a next-generation sequencer (Figure 1).

The NULISAseq assay is fully automated on the ARGO HT instrument. The output of the ARGO system is a single purified library ready to load onto an external Illumina system for sequencing. The results of the sequencing run will be a FASTQ file that can then be uploaded into the ARGO Control Center (ACC) software for demultiplexing, normalization and subsequent data analysis (Figure 2).

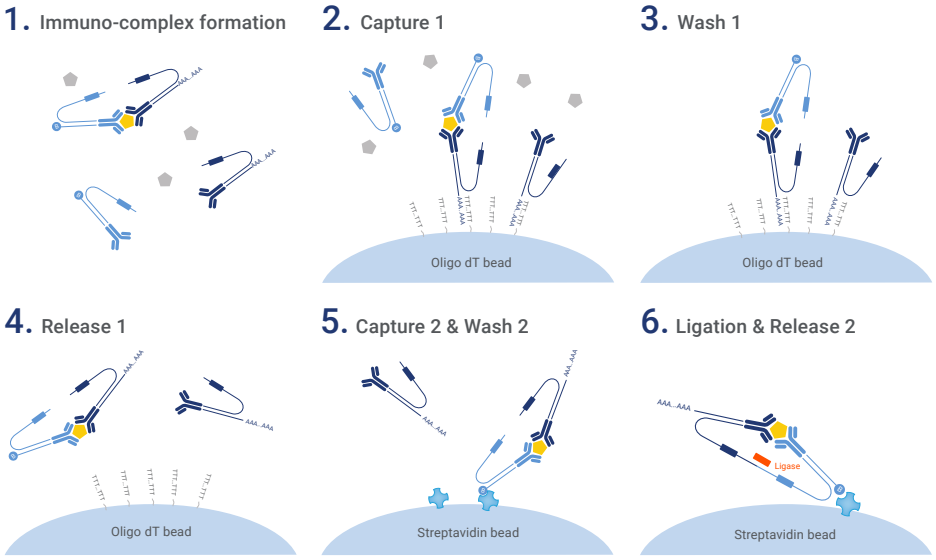


**Figure 1.** NULISA workflow.

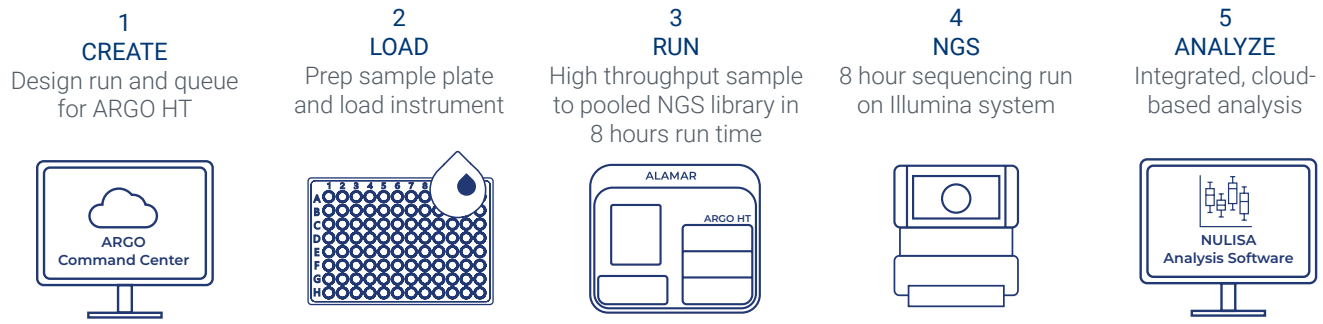


**Figure 2.** ARGO HT workflow with <30 minutes total hands-on time.



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## Next Generation Sequencing (NGS)

### NULISAseq NGS Library Creation

During the NULISAseq multiplex assay workflow, target specific barcodes (TMI) are brought into proximity and a double-stranded DNA oligonucleotide is ligated between these containing a sample-specific molecular identifier (SMI) that is specific for the well of the assay plate (Figure 3). The resulting library of synthetic DNA encoded with sample and target specific information is then PCR amplified, purified and pooled into one well on the detection plate. The entire process is automated on the ARGO HT instrument and the only user-required step is to transfer the pooled library from the plate into a DNA Low binding tube.

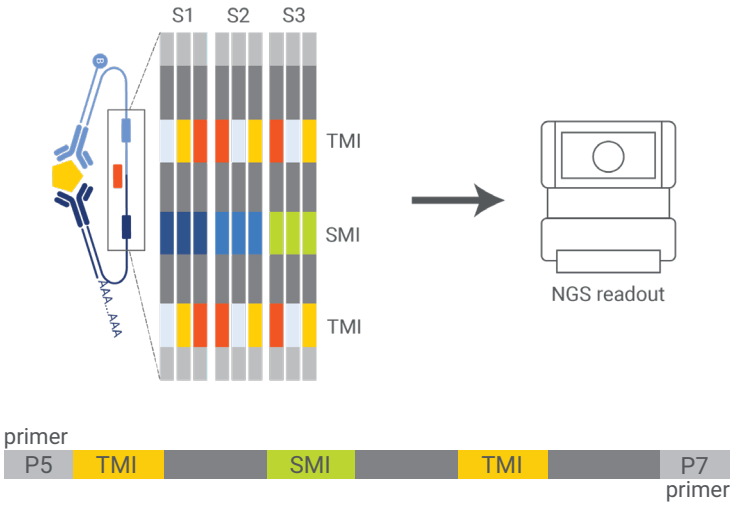


**Figure 3.** NULISA readout by quantification of reporter DNA levels by NGS. Antibody-specific barcode sequences TMI and site-specific barcode sequence SMI are shown along with detail on library fragment including P5 and P7 primer binding sites.

### Selecting a Compatible Illumina System and Sequencing Kit

Illumina NGS systems utilize sequencing by synthesis (SBS) technology for massively parallel analysis of libraries of short DNA fragments. One NULISAseq library can be sequenced per lane of a flow cell with the requirement of at least 400 million reads/library. The table below describes the compatible Illumina sequencers and recommended kits to achieve this level of sequencing depth.

### Choosing the Best Illumina System Based on Throughput and Costs

The NextSeq 1000/2000 in combination with the P2 flow cell is ideal for a single flow cell and with the short sequencing recipe (see below) two sequencing runs can be completed per day. If running the ARGO HT at maximum capacity (3 NULISAseq runs/24 hours) we recommend using a NovaSeq 6000 or NovaSeq X in order to reduce time and costs. Especially for projects covering a large number of plates the option to sequence 8 libraries in one run on the NovaSeq X allows reducing costs significantly.

| Sequencer | Kit | Lanes |
|---|---|---|
| NextSeq 550 | TG NextSeq™ 500/550 High Output Kit v2.5 (75 cycles) | 1 |
| NextSeq 1000/2000 | NextSeq 1000/2000 P2 Reagents v3 (100 cycles) | 1 |
| NextSeq 1000/2000 | NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (100 Cycles) | 1 |
| NovaSeq 6000 | NovaSeq 6000 SP Reagent Kit v1.5 (100 cycles) | 2 |
| NovaSeq X | NovaSeq X Series 1.5B Reagent Kit (100 Cycle) | 2 |
| NovaSeq X | NovaSeq X Series 10B Reagent Kit (100 Cycle) | 8 |

**Table 1.** Illumina sequencer models with recommended sequencing kit and number of lanes per flow cell allowing the respective number of libraries to be sequenced in parallel

### Illumina Sequencing Parameters

The following sequencing parameters should be selected for analysis of NULISAseq NGS libraries

- Single-end sequencing
- Read length = 34 bases
- No indices, no de-multiplexing done at the end of run
- Read depth: 400 M reads

### Library Dilution

Prior to sequencing, the library should be quantified using Qubit and diluted to 2 nM with molecular biology grade water.

Calculation for library dilution after Qubit quantification:

$$\frac{\textit{Concentration on Qubit (ng/µL)}}{\textit{77800 (MW, } \frac{g}{mole}\textit{)}} \; X \; \frac{\textit{1,000,000 µL}}{\textit{L}} \; = \textit{library concentration (nM)}$$

The recommended loading concentration varies by sequencer model used and SBS chemistry – please reach out to support@alamarbio.com for guidance.

### Illumina Sequencing Recipes

A custom recipe (or sequencing protocol) is required to process the NULISAseq library. This recipe is specific to the sequencer model and the flow cell being used and the file needs to be loaded on the sequencer and referenced when setting up the run.

### Analysis of Results

After sequencing, the compressed FASTQ file can then be uploaded into the ARGO Command Center (ACC) software by either using a local file, or a direct BaseSpace link.

Additional details are provided in the ARGO HT User's Guide.

Please send an email to support@alamarbio.com to request the sequencing recipe and run setup information for your specific setup.

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